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Effect of Preslaughter Events on Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in Market-Weight Turkeys

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The effects of events which occur prior to slaughter, such as loading, transport, and holding at an abattoir, on the prevalence of *Campylobacter* species, including *Campylobacter jejuni* and *Campylobacter coli*, were examined. Cloacal swabs from market-weight turkeys in each of five flocks were obtained on a farm prior to loading (time 1; 120 swabs per flock) and after transport and holding at the abattoir (time 2; 120 swabs per flock). A statistically significant increase in the overall prevalence of *Campylobacter* spp. was observed for cloacal swabs obtained from farm 3 following transport ($P < 0.01$). At time 2, an increase in the prevalence of *C. coli* was also noted for cloacal swabs from farms 3, 4, and 5 ($P < 0.01$). Neither the minimum time off of feed nor the distance transported from the farm to the abattoir was correlated with the increase in *C. coli* prevalence. Similarly, responses to an on-farm management questionnaire failed to detect any factors contributing to the observed changes in *Campylobacter* sp. prevalence. A SmaI macrorestriction analysis of *Campylobacter* sp. isolates recovered from flock 5 indicated that *C. coli* was more diverse than *C. jejuni* at both time 1 and time 2 ($P < 0.01$), based on a comparison of the Shannon indices of diversity and evenness.

Human food-borne campylobacteriosis causes nearly 2 million cases of food-borne illness annually, resulting in ~10,000 hospitalizations and ~100 deaths each year (18). Of the three thermotolerant *Campylobacter* species, *Campylobacter jejuni*, *C. coli*, and *C. lari*, *C. jejuni* is the primary human pathogen. *C. coli* is a commensal of hogs, is occasionally reported in poultry, and is also a human pathogen (1, 14, 31, 32). *C. lari*, which is present in poultry, hogs, and shellfish, is infrequently associated with human illness (12). The consumption of contaminated undercooked poultry is a major risk factor for human *Campylobacter* infections (1, 11, 20, 29). The 1997 USDA Food Safety and Inspection Service (FSIS) young turkey baseline study detected *Campylobacter jejuni/coli* on 90% of turkey carcasses (34). In that national study, FSIS protocols did not require the identification of *C. jejuni* and *C. coli* to the species level. Thus, reducing the prevalence of *Campylobacter* spp. in live birds entering abattoirs may ultimately reduce the occurrence of human campylobacteriosis.

Feed withdrawal, catching, crating, and transport occur within 24 h of slaughter and may influence the intestinal carriage of food-borne pathogens such as *Campylobacter* spp. Feed withdrawal immediately prior to live hauling evacuates the crop, minimizes the gastrointestinal contents, and thereby reduces fecal contamination of both turkey and broiler carcasses (6, 17, 35). After ~4 h of feed withdrawal, birds may reflexively peck at fecal-contaminated litter, thus increasing crop contamination (6). In a study of broilers, the percentage of crops harboring *Campylobacter* spp. increased significantly after feed withdrawal (62.4%) compared to the baseline level (25%; $P < 0.001$) (4). In the same study, the prevalence of *Campylobacter* spp. in crops (62%) exceeded that of *Campylobacter*-positive ceca (4%; $P < 0.001$) after transport and holding, which suggested that the crop may be a critical control point for reducing the entry of *Campylobacter* into the abattoir (4). The effects of feed withdrawal may be age related, as demonstrated by Northcutt et al., who reported that feed withdrawal increased *Campylobacter* levels on carcasses of younger birds (42 days), with older animals (49 and 56 days) being unaffected (23).

Whereas the physical exertion during catching may increase intestinal peristalsis in poultry and augment the excretion of food-borne pathogens, the inactivity while cooped during transport slows gut motility (17). Contacts of birds with dirty crates as well as with excrement contaminate the feet and feathers of colonized birds as well as those of previously uncontaminated cagemates (23, 27). The idea that transport crates are a source of contamination was based on a comparison of genotypes of *Campylobacter* isolates recovered from carcasses with those recovered from transport crates. Crates may already be contaminated prior to loading (21, 27). In a previous study, confinement in a crate significantly increased the prevalence of *Campylobacter* on broiler carcasses, some of which originated from *Campylobacter*-negative flocks (27). In that study, the contamination may have originated from either workers crating the birds prior to transport or workers shackling the birds at the abattoir (27).

Stern et al. reported that transportation and holding increase *Campylobacter* carcass contamination in broilers (28). In their study, *Campylobacter* was detected on 12.1% of carcasses of broilers that were slaughtered on the farm (average = $\log_{10}$ 2.71 *Campylobacter* spp. per carcass). After transport and slaughter, 56% of carcasses harbored *Campylobacter* at higher levels ($\log_{10}$ 5.15 CFU per carcass; $P < 0.05$). Interestingly, no such increases were noted for *Campylobacter* spp. recovered

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from the ceca. In addition, Whyte et al. (38) reported that the numbers of *Campylobacter* present in feces of broilers increased significantly after transportation.

Limited information is available detailing the effects of events which occur prior to slaughter on the prevalence of food-borne pathogens in turkeys. Therefore, based on studies of broilers, the primary goal of this study was to determine if feed withdrawal, transport, and holding at the abattoir collectively influence the prevalence of *C. jejuni* and *C. coli* populations in market-weight turkeys.

(Portions of this study were presented at the annual convention of the Midwest Poultry Federation, Minneapolis, Minn., 15 to 17 March 2004.)

MATERIALS AND METHODS

Farm selection. Five Midwestern premises (farms 1 through 5) were selected on the basis of their scheduled delivery of market-weight turkeys to the abattoir during the summer months of May through August 2003, when sampling was conducted. Four of the five farms utilized a two-stage production system with separate brooder and grow-out houses (~10,000 birds per house). The fifth farm utilized a three-stage production system in which ~6-week-old birds remained in the first grow-out house until ~12 weeks of age, when they were transferred to a second grow-out house until achieving market weight (~40 lbs).

Live hauling. All producers used the same company for live hauling to a single commercial abattoir. Estimates of the perimarketing interval were based on the drivers' logs, which included the times for loading (catching and crating) at the farm, transport, a resting interval at the processing plant, and the plant's record of the time of slaughter.

Cloacal swabs. Cloacal swabs were obtained at each farm within 12 h of the beginning of catching and loading into transport cages (time 1; $n = 120$ per farm) and again at the slaughter facility prior to slaughter (time 2; $n = 120$ per farm). Six-inch cotton-tipped applicators (Harwood Products, Guilford, Maine) were inserted approximately 3 inches into the cloaca, with care taken to avoid contact with the surrounding feathers and skin. The wooden applicator was broken in half and the upper half was discarded as each swab was placed into a 16- by 125-mm round-bottomed polystyrene tube (Becton-Dickinson, Franklin Lakes, N.J.) containing 13.5 ml blood-free enrichment broth (BFEB), allowing for the requisite headspace air to take up 16 to 17% of the tube's total capacity (33). Tubes were transported to the laboratory, where they were immediately incubated (24 h, 42°C, ambient atmosphere). BFEB, which incorporates activated charcoal as an oxygen quencher, has been shown to be equivalent to the U.S. Food and Drug Administration-approved protocol for *Campylobacter* isolation (33).

Viscera sampling. Ceca and crops were analyzed for farms 4 and 5. At the slaughterhouse, organs were placed individually into sterile WhirlPak bags (Nasco, Ft. Atkinson, Wisc.), transported back to the laboratory at ambient temperature, refrigerated within ~6 h of collection, and processed the following morning.

Isolation and identification of *Campylobacter* spp. For cloacal swabs, after enrichment in BFEB (24 h, 42°C), an aliquot (50 μl) was streaked onto Campy cefex agar (30) and incubated (48 h, 42°C) microaerobically (5% O_2, 10% CO_2, 85% N_2) in a three-gas incubator (Forma Scientific, Marietta, Ohio).

For crops, sterile phosphate-buffered saline (10 ml) was pipetted into each crop and the contents mixed by hand to achieve a homogeneous suspension. An aliquot of this suspension (1 ml) was placed in BFEB (12.5 ml) and incubated (24 h, 42°C), after which an aliquot (100 μl) was subcultured onto Campy cefex agar and incubated (48 h, 42°C) microaerobically as described above.

The contents from both ceca (~5 g) were squeezed into buffered peptone water (90 ml) and mixed, and an aliquot (1 ml) was placed in BFEB (12.5 ml) and incubated (24 h, 42°C). An aliquot (100 μl) was subcultured onto Campy cefex agar plates (48 h, 42°C) and incubated microaerobically.

Species of *Campylobacter* were determined as follows. For cloacal swabs and viscera, three presumptive *Campylobacter* colonies from each Campy cefex agar plate (flat, shiny, and mucoid, with a pink hue) were randomly picked to ensure an even representation of colony types. The colonies were subcultured on brain heart infusion agar (Becton Dickinson, Sparks, Md.) supplemented with 0.6% yeast extract and 10% defibrinated sheep blood and then were incubated microaerobically (24 h, 42°C).

Presumptive colonies were identified by use of a multiplex PCR assay for *C. jejuni* and *C. coli*, as described previously (5). Templates for PCR were initially prepared (farms 1 and 2) with an InstaGene Matrix (Bio-Rad Laboratories, Hercules, Calif.) according to the manufacturer's directions. For reasons of efficiency, cost of template preparation, number of samples processed, and improved template recovery, DNA templates were ultimately prepared by using boiled sterile distilled water (farms 3, 4, and 5). For the aqueous method of template preparation, bacteria were harvested from a 2- by 4-mm area of growth on blood agar plates, placed in 50 μl of sterile distilled water, and incubated (10 min, 100°C), and the lysate was then centrifuged (11,000 rpm, 3 min; IEC Micromax, Needham Heights, Maine). Multiplex PCR was performed as previously described (5), using either 20 μl of the InstaGene supernatant (farms 1 and 2) or 5 μl of the water template preparation (farms 3 to 5). Presumptive *Campylobacter* colonies that were not identified as either *C. jejuni* or *C. coli* were further subjected to a PCR for the *Campylobacter* genus and then a PCR for *C. lari* as described previously (13), using a 5-μl template. All isolates confirmed as *Campylobacter* spp. were stored in brain heart infusion broth (Becton Dickinson, Sparks, Md.) with 20% glycerol (−80°C).

PFGE. Farm 5 was randomly selected for a pulsed-field gel electrophoresis (PFGE) analysis of isolates recovered at time 1 and time 2. A macrorestriction analysis of the *C. jejuni* ($n = 88$ isolates) and *C. coli* ($n = 71$) isolates utilized SmaI and was conducted with a Chef Mapper (Bio-Rad Laboratories, Hercules, Calif.) as described previously (25). *Campylobacter coli* ATCC 33559, which originated from swine feces, served as the positive control. The lambda *c*I857 DNA ladder (0.05 to 1 Mb) was used as the size standard (Bio-Rad Laboratories, Hercules, Calif.).

Analysis of PFGE restriction enzyme digestion profiles. GelCompar II v. 3 software (Applied Maths, Kortrijk, Belgium) was employed for band analysis and dendrogram construction. Patterns were normalized according to the lambda size standard, with a 1% band tolerance using Dice similarity coefficients, as described previously (19). To ensure gel-to-gel consistency for band comparisons, we included the *C. coli* reference standard thrice in each gel.

Statistical analysis. Chi-square analysis was used to compare the prevalence of *Campylobacter* at time 1 (on-farm) and time 2 (after transport). P values of <0.01 were considered significant.

The Shannon indices of diversity (H') and evenness (E) were used to compare the diversities of *C. jejuni* and *C. coli* isolates (16).

The Shannon index of diversity (H') is defined as follows: $H' = -\Sigma p_i \ln(p_i)$, where $p_i = n_i/N$ and is the proportion of a strain found in an isolate. The Shannon index of evenness was used to measure the abundance of isolates and is defined as follows: $E = H'/\ln(S)$, where S is the number of strains.

The t test was used to statistically compare the H' values of *C. jejuni* and *C. coli* isolates recovered at time 1 and time 2, with significance set at P values of <0.001, as described previously (16). In the absence of a formula for calculating variance, t tests were not done for the E values.

Farm management questionnaire. A farm management questionnaire was used in a follow-up telephone interview of turkey growers to determine if any management practices were associated with the prevalence of *Campylobacter* at the time of slaughter. The questionnaire surveyed, for example, the source of poults, the use of growth promoters, the frequency of top dressing litter changes, vaccine strategies, the use of coccidiostats, health problems associated with the flock, and the use of chlorinated water. The interviewer was unaware of individual farm *Campylobacter* prevalence data when the questionnaire was administered.

RESULTS

Duration of preslaughter events. The average estimated perimarketing interval, or minimum time off feed, was 7.94 h for the five premises, as shown in Table 1. Although the loading times (mean, 0.79 h) were similar for the five flocks, the transport time did vary from 0.25 h (flock 2) to 3 h (flock 1). Birds rested in the holding shed, where they were cooled by industrial-size fans, for an average of 6 h. Coincidentally, flock 2, which had the shortest transport time, had the longest interval (10 h) in the holding shed.

***Campylobacter* sp. prevalence.** As shown in Fig. 1, the baseline prevalence of *Campylobacter* spp. in turkeys from the five premises ranged from 65% to 90% on-farm prior to transport (time 1) and from 66% to 95% at the slaughter facility (time 2).

TABLE 1. Summary of times for loading, transport, and holding and minimum overall time off feed (feed withdrawal interval) for turkeys sampled from each of five premises

Farm no.	Loading time (h)	Transit time (h)	Holding time (h)	Minimum time off feed (h)
1	0.76	3	4.38	8.14
2	0.78	0.25	9.75	10.78
3	0.75	0.75	5.1	6.6
4	0.73	1	4.87	6.6
5	0.92	1	5.7	7.62
Mean ± SD (h)	0.79 ± 0.08	1.2 ± 1.05	5.96 ± 2.17	7.94 ± 1.72
Range (h)	0.73–0.92	0.25–3	4.38–9.75	5.6–11

Chi-square analysis indicated a statistically significant increase in the prevalence of *Campylobacter* spp. in flock 3 between time 1 (65%; on-farm) and time 2 (81%; after transport [$P < 0.01$]). The overall prevalence of *Campylobacter* spp. did not differ prior to (time 1) and after (time 2) transport for the remaining four of the five flocks examined.

As shown in Fig. 2, when *Campylobacter* isolates were identified to the species level, significant differences emerged. For the isolates from flocks 3, 4, and 5, an increase in the prevalence of *C. coli* was noted for cloacal swabs taken at the holding shed at the slaughter plant (time 2) compared to the on-farm baseline data (time 1). For flock 5, the prevalence of *C. coli* exceeded that of *C. jejuni*. This coincided with a lower prevalence of *C. jejuni* at time 2 ($P < 0.01$). Birds from which both *C. jejuni* and *C. coli* were isolated were scored as concurrently positive and were grouped separately from birds from which either *C. jejuni* or *C. coli* was exclusively recovered. A significant increase in the frequency of concurrently positive birds between the two sampling times was observed at time 2 for farms 3 and 4 ($P < 0.01$) (Fig. 2).

The majority of isolates recovered from cloacal swabs were *C. jejuni* (69%; 735/1,066), followed by *C. coli* (30.5%; 325/1,066) and *C. lari* (0.6%; 6/1,066). In contrast, as shown in Table 2, *C. coli* was the predominant species recovered from limited sampling of the crop (61/84; 72.6%) and the cecum (96/96; 100%).

Farm management. As summarized in Table 3, the flocks differed regarding the source of poults, the mill providing feed, the type of litter, the source of water in the turkey houses, water chlorination, vaccine regimens, and biosecurity practices. All flocks used growth promoters, at minimum moved birds from a brooder house to a grow-out house (two-stage production), top dressed the litter with the introduction of each flock, recorded sparrows or starlings in the houses, and used coccidiostats. However, with this small sample, no single factor emerged to explain the observed shift in *C. jejuni* and *C. coli* populations between the two sampling intervals.

PFGE. Isolates from flock 5 were examined by PFGE to compare the relative diversities of *C. coli* and *C. jejuni* recovered at time 1 ($n = 80$) with those of organisms recovered after transport and holding at time 2 ($n = 79$). As shown in Fig. 3 (top panel), for the two time points combined, the *C. jejuni* isolates ($n = 88$) exhibited seven restriction enzyme patterns (arbitrarily designated J1 to J7). Profile J1 was exhibited by 79.5% of the isolates (31 of 39) at time 1 and by 82% of the isolates (40 of 49) at time 2. Thus, the J1 profile was represented more frequently than the remaining *C. jejuni* patterns (J2 to J7). As shown in Fig. 3 (bottom panel), for time 1 and

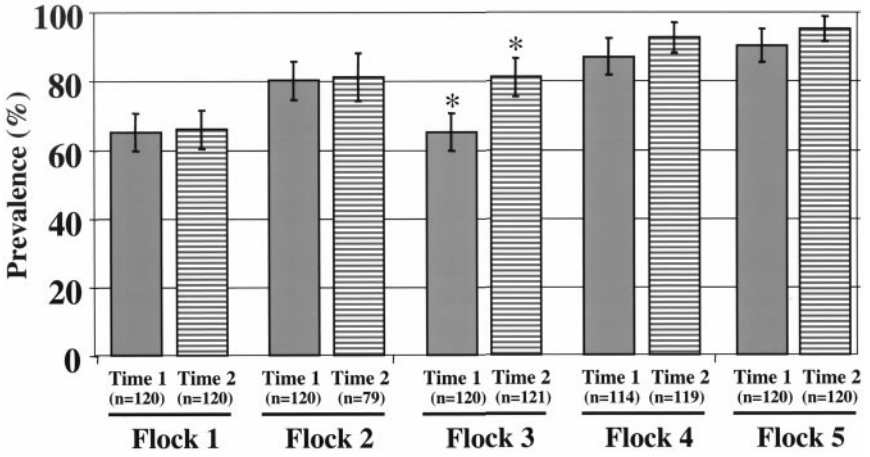

FIG. 1. Prevalence of *Campylobacter* spp. in five Midwestern turkey flocks, as determined by use of cloacal swabs. Time 1 represents the prevalence on-farm prior to loading into transport cages. Time 2 represents the prevalence after transport and holding (at the abattoir). Error bars indicate 95% confidence intervals (precision of prevalence estimates). *, significant difference ($P < 0.01$) in the prevalence of *Campylobacter* spp. between the two sampling times.

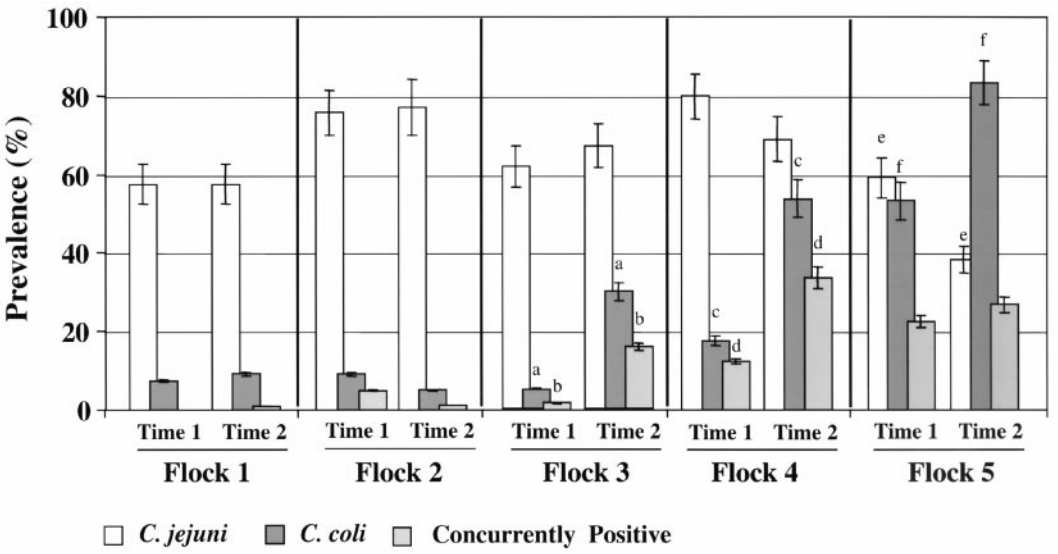

FIG. 2. Prevalence of *C. jejuni*, *C. coli*, and concurrently positive turkeys in five turkey flocks, as determined by use of cloacal swabs. Birds from which both *C. jejuni* and *C. coli* were isolated were scored as "concurrently positive" and were grouped separately from birds from which either *C. jejuni* or *C. coli* was exclusively recovered. Time 1 represents the on-farm prevalence. Time 2 represents the prevalence after transport and holding at the abattoir. Error bars indicate 95% confidence intervals (precision of prevalence estimates). Error bars with the same letter indicate a significant difference ($P < 0.01$) in the prevalence of *Campylobacter* between the two sampling times.

time 2 overall, the *C. coli* isolates ($n = 71$) exhibited 17 patterns, or nearly 2.4 times more unique profiles (C1 to C17), than a comparable number of *C. jejuni* isolates ($n = 88$). In addition, the *C. coli* isolates collected at time 1 ($n = 41$ isolates) exhibited more patterns ($n = 15$) than the isolates collected at time 2 ($n = 30$ isolates), which exhibited eight patterns.

As summarized in Table 4, a comparison of the Shannon indices of diversity (H') and evenness (E) affirmed that *C. coli* had more diversity among the isolates, and thus more strains, than did *C. jejuni* at both time 1 and time 2. For the index of diversity (H'), this difference was statistically significant ($P < 0.001$). Furthermore, the *C. coli* isolates recovered at time 1 were more diverse than those collected at time 2 ($P < 0.001$), based on a comparison of H' values, which may reflect the smaller number of *C. coli* recovered at time 2. In contrast, there was no difference in the diversity (H') of *C. jejuni* at time 1 ($n = 39$ isolates [four strains]) and that at time 2 ($n = 49$ isolates [six strains]; $P > 0.4$).

TABLE 2. Recovery of *C. jejuni* and *C. coli* from the crop and cecum based on limited sampling of farms 4 and 5

Sample type or organism	Recovery of organism (no. of samples/total [%])		
	Farm 4	Farm 5	Total
Crop			
C. jejuni	11/50 (22)	0/34 (0)	11/84 (13)
C. coli	33/50 (66)	28/34 (82.35)	61/84 (72.6)
Negative[a]	6/50 (12)	6/34 (17.7)	12/84 (14.3)
Cecum			
C. jejuni	2/47 (4.3)	0/49 (0)	2/96 (2)
C. coli	47/47 (100)	49/49 (100)	96/96 (100)
Negative[a]	0/47 (0)	0/49 (0)	0/96 (0)

[a] No organisms were detected.

DISCUSSION

Feed withdrawal, catching, crating, live hauling, and resting in holding sheds at the slaughterhouse are routinely practiced in the turkey industry. In this study of five independently operated commercial turkey farms, we compared the prevalence of *Campylobacter* spp. in turkey cloacal swabs collected on-farm (time 1) and after transport to the abattoir (time 2). Turkeys had no access to feed for an average of nearly 8 h, based on the interval from the beginning of loading to the time of slaughter. This was the minimum interval, since the exact time that feed was withdrawn varied for each farm, with some premises allowing access to feed until loading began.

The overall prevalence of *Campylobacter* spp. in cloacal swabs from tom turkeys sampled during the summer months both on-farm (time 1; 65 to 90%) and immediately prior to slaughter (time 2; 66 to 95%) was comparable to that reported for the southeastern Atlantic states, where *Campylobacter* spp. were frequently isolated from cecal droppings of hens (70%) and toms (80%) (7).

Compared to the on-farm baseline (time 1), there was a significant increase in the prevalence of *Campylobacter* spp. after transport and holding (time 2) in cloacal swabs from turkeys in flock 3. The fact that no other flock exhibited a change may be attributed to the already high prevalence of *Campylobacter* spp. and the resultant need for sampling more birds per flock to detect a statistically significant shift. The impact of perimarketing events, reflected in the dynamics of gut microflora, on *Campylobacter* sp. prevalence in broilers has been noted earlier (28, 38). For example, the prevalence and mean counts of *Campylobacter* spp. on carcasses, but not in ceca, increased for broilers slaughtered after feed withdrawal and transport compared with birds slaughtered on-farm (28). In addition, mean counts of *Campylobacter* spp. in feces increased significantly after the transportation of broilers com-

TABLE 3. Summary of on-farm management practices for the farms in this study

Parameter	Description or practice for indicated farm				
	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Turkeys					
Hatchery	A	B	C	D	C
Poult strain	HyBrid	HyBrid	HyBrid	BUTA	HyBrid
Growing program	Two-stage	Two-stage	Two-stage	Two-stage	Three-stage
Feed mill	E	F	G	E	H
Growth antibiotic	Virginiamycin	Bacitracin	Virginiamycin	Bacitracin and Virginiamycin	Virginiamycin
Dose (g/ton)	20	48 (9–12 wks), 42 (12–15 wks), 30 (15–18 wks)	20	50	20
Age fed	3 wks to market		9 wks to market	1 to 39 days (bacitrocin), 40 day to market (virginiamycin)	8 wks to market
Water					
Water source	Farm well	Farm well	Farm well	Farm well	City water
Chlorination	No	No	No	Yes	Yes
Water lines cleaned (time period)	2 mos	1 yr	Unknown	Unknown	Each flock
Litter					
Litter type	Pine shavings, oat hulls	Pine/soft wood shavings	Wood shavings	Wood shavings	Shavings
Time since last change	Scrape and top dress prior to each flock	1 yr	2 yrs (top change)	Fresh for flock	Top dress each flock
Animals					
Animals in house	No	No	Yes (dogs)	No	No
Mice/rats	Yes	Yes (mice)	Occasional (mice)	No	Occasional
Birds (sparrows)	Yes (starlings)	Yes	Yes	Yes (a few)	Yes
Other livestock on farm	No	Cattle	No	No	No
Flock health					
Vaccines used	Yes	Yes	None	Yes	Yes
Hemorrhagic enteritis virus	Yes			Yes	Yes
Pasteurella		Yes			Yes
Bordetella		Yes		Yes	Yes
Coccidiostat used	Yes	Yes	Yes	Yes	Yes
Diseases	*E. coli*	Nothing major	Crop mycosis	None	None
Treatment	Chlortetra-penicillin, cycline	$CuSO_4$ (brooder) Oxytetracycline sulfa (12.5%)	No Oxytetracycline (3 wks)		Penicillin (prophylaxis)
Age treated	6–7 wks	1, 2, and 10 wks	See above		8 wks
Biosecurity					
Foot bath required	No	Yes	No	No	No
Disinfectant		Tektrol			
Boot change	Yes	Yes	No	No	Yes
Clothes change	No	No	No	No	No

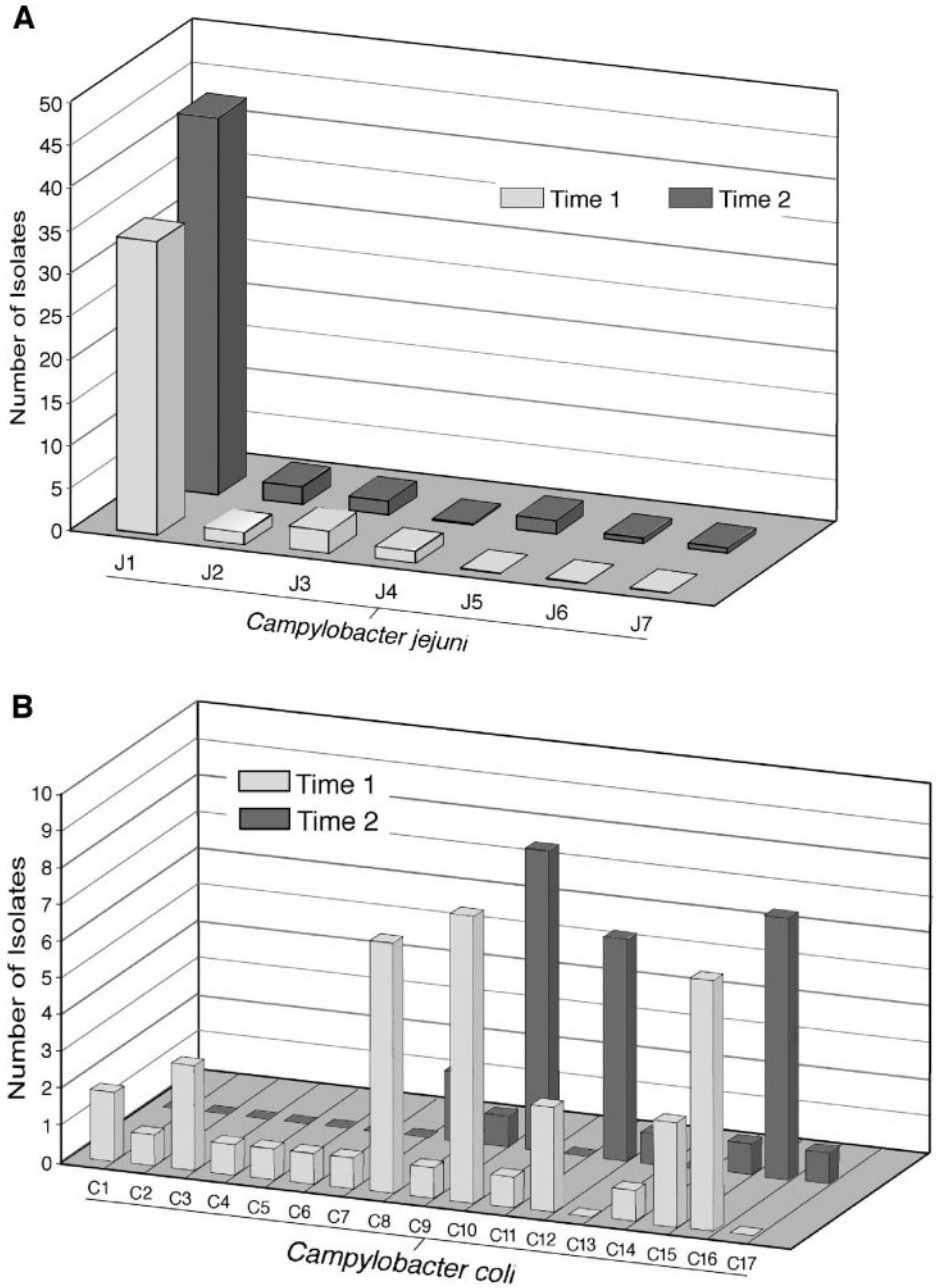

FIG. 3. Distribution of SmaI macrorestriction patterns of *Campylobacter* spp. isolated from cloacal swabs from turkeys in farm 5. Cloacal swabs were collected prior to (time 1) and after (time 2) feed withdrawal, transport, and holding at the abattoir. The frequencies of each pattern for time 1 (on-farm baseline) and time 2 (at the abattoir) are indicated. (A) Seven profiles (J1 to J7) were exhibited by *C. jejuni* isolates ($n = 88$). (B) Seventeen profiles (C1 to C17) were exhibited by *C. coli* isolates ($n = 71$). Calculation of the Shannon index of diversity also indicated that *C. coli* was more diverse than *C. jejuni* at both sampling times.

pared with pretransport levels, although the overall *Campylobacter* sp. prevalence was unchanged (38).

In the present study, the prevalence of *C. coli* increased significantly in cloacal swabs taken after the transportation of turkeys originating from three of the five premises compared with the baseline on-farm sampling at time 1 ($P < 0.05$; flocks 3, 4, and 5). These events coincided with a significant increase in concurrent infections of *C. jejuni* and *C. coli* for two of the premises ($P < 0.05$; flocks 3 and 4). The fact that *C. coli* was the predominant species recovered after enrichment in a limited study of ceca (96 of 96 [100%]) and crops (61 of 84 [62%]) suggests that these sites may serve as reservoirs for carcass contamination (36). The physiological consequences of feed withdrawal, crating, transport, and holding may have altered the gut microflora and thus preferentially favored the replication and survival of *C. coli* rather than *C. jejuni* at detectable levels. For the crops of broilers during the first 12 h of feed withdrawal, a decline in *Salmonella* and lactic acid bacteria

TABLE 4. Comparison of *C. jejuni* and *C. coli* isolates recovered at time 1 and time 2 by their Shannon indices of diversity (H') and evenness (E)

Parameter	Value for *C. jejuni*		Value for *C. coli*	
	Time 1	Time 2	Time 1	Time 2
No. of strains (S)	4	6	15	8
Total no. of isolates (n)	39	49	41	30
Diversity index (H')	0.72071	0.75664	2.3684	1.72431
Variance of H'	0.0236	0.0274	0.0108	0.0101
Evenness index (E)	0.51988	0.42229	0.8746	0.82922

with a concurrent increase in the crop pH has been noted (9). For the ceca of broilers, in contrast, since lactic acid bacteria are present in smaller numbers, their decline generates a modest pH increase only after 12 h of feed withdrawal, with a minimal impact on either *Campylobacter* or *Salmonella* (3, 10).

No specific on-farm management practices were clearly associated with the shift in the *C. jejuni* and *C. coli* populations, which may have been due to the small number of flocks surveyed. The five premises were independently operated and employed similar overall industry practices. Although three of the premises did not use chlorinated water, this was not associated with the observed change in *Campylobacter* sp. prevalence either pre- or posttransport. Feed withdrawal compounded with the physical exertion of catching and loading may initially favor voiding of the intestinal tract, thus reducing carcass contamination. However, the limited movement of turkeys after crating slows gut motility (17). For this study, birds were held in transport crates for an average duration of nearly 8 h, which would have favored gut stasis and the subsequent retention of digesta (4). Turkey ceca harbor 2.7×10^6 *Campylobacter* cells per gram of contents, which surpasses the amounts in other segments of the intestine by up to 5 orders of magnitude (15, 36, 39). Thus, *C. coli* from the cecum may have been voided into the cloaca, resulting in the observed increase after transport. Although physiological changes in the crops (9) and ceca (10) of broiler chickens have been reported after feed withdrawal and confinement in a transport crate, limited information is available for turkeys. Alternatively, since the individual turkeys swabbed on-farm (time 1) differed from those sampled posttransport (time 2), it is possible that the difference merely reflected a sampling bias, despite the random selection of turkeys.

Few studies have delineated the *Campylobacter* species recovered from turkeys, perhaps assuming that *C. jejuni* represents the majority of isolates. For example, the 1997 USDA National Young Turkey Microbial Baseline survey reported the presence of *Campylobacter jejuni*/*C. coli* on 90.3% of carcasses and did not further identify isolates to the species level (34). Although regarded as an intestinal commensal of swine, *C. coli* has been isolated from up to 25% of turkeys (2, 14, 22) and up to 20% of broiler chickens (26). In the present study, *C. coli* was frequently recovered from turkeys, although hogs were not raised on any of the premises. In a survey of *Campylobacter* spp. in two turkey processing plants in the upper Midwestern United States, Logue et al. (14) reported that 34.9% of carcasses overall harbored *Campylobacter* spp., with the contamination rates being similar for the two slaughterhouses. In that study, the proportion of *C. jejuni* and *C. coli* on turkey carcasses varied from plant A (51.6% and 40.5%, respectively) to plant B (76.8% and 4.6%, respectively).

Farm 5 was randomly selected for PFGE analysis to gauge the genetic diversity of the isolates. Overall *C. jejuni* exhibited proportionately fewer macrorestriction profiles by PFGE than did *C. coli*. The Shannon indices of diversity and evenness also indicated that *C. coli* was more diverse than *C. jejuni*. It is possible that *C. jejuni* encountered in the field may have been preferentially more sensitive to the antimicrobials incorporated in the selective enrichments, thus favoring the recovery of a broader range of *C. coli*. The fact that a single profile (J1) was overrepresented among the seven unique profiles of *C. jejuni* may reflect a long-standing colonizing strain introduced early into the flock (24, 37) or preferential strain survival after selective enrichment (21). Limited genomic diversity and thus genetic stability were suggested earlier for *C. jejuni* isolated from turkey flocks raised simultaneously on individual farms (2) and from broiler chicken carcasses purchased from a single producer (8). In contrast, Rivoal et al. attributed the multiple genotypes of *C. coli* (4 strains) and *C. jejuni* (11 strains) within a broiler chicken flock to either multiple origins or genetic drift within the *Campylobacter* population (26). The multiple genotypes exhibited by the 71 isolates of *C. coli* from a single farm in this study may reflect its survival advantage in the intestine of the healthy turkey (37).

This constitutes the first report of a shift in *C. jejuni* and *C. coli* populations in turkeys associated with perislaughter events, including catching, crating, transport, and resting at the abattoir. These observations concur with a previous analysis of the crop and cecum and suggest that the overall gut microbiota is impacted by feed withdrawal, crating, transport, and holding. Whether these events favor the selection of more virulent strains or of bacteria with enhanced antimicrobial resistance is unknown.

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